Effect of a carnivorous diet on the lipids, fatty acids and condition of Antarctic krill, *Euphausia superba*

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**Abstract:** Krill are thought to be predominantly herbivorous, but a heterotrophic diet might be crucial for their growth and survival. To compare the influence of herbivory and carnivory on krill we conducted a nine month feeding trial. We examined lipid composition of the hepatopancreas, abdomen and remaining body portions of krill fed diatoms at bloom condition levels, and diatoms with the addition of pellets or minced clam meat to simulate a partly carnivorous diet. Mortality, dry mass and lipid content were similar among treatments. We examined lipid class and fatty acid profiles, with emphasis placed on the ratio of storage (triacylglycerol) to structural (polar lipid) lipid and key essential omega 3 polyunsaturated fatty acids: 20:5ω3 and 22:6ω3. The triacylglycerol : polar lipid ratio increased in krill fed on the mixed diet as did the 20:5ω3 : 22:6ω3 ratio. Overall these findings indicate that provision of clam in the diet improved krill condition, and further suggest that carnivory may aid krill growth in the wild under certain environmental conditions.

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**Introduction**

There is a wealth of information available on the Antarctic krill *Euphausia superba* Dana, although some cardinal questions about its life history are still under intense discussion. Various hypotheses, sometimes conflicting, prevail on the overwintering strategies of *E. superba*, due, in part, to limited availability of winter data. Studies suggest that krill suppress metabolism over winter to cope with reduced primary productivity (Cullen *et al.* 2003). Utilization of a range of food sources such as detritus, bacteria or metazoans (omnivory or carnivory) has been suggested as another strategy to avoid starvation due to the lack of phytoplankton during the dark season (Clarke 1980, Ikeda & Dixon 1982, Kawaguchi *et al.* 1986, Perissinotto *et al.* 2000, Atkinson *et al.* 2002).

Little experimental evidence is available on the impact of animal food (carnivory) on the condition of krill. Krill are pelagic animals which live in aggregations and feed on suspended material, so their natural diet must include a variety of items of both plant and animal origin during all seasons. Cripps & Atkinson (2000) detected a strong increase in polyunsaturated fatty acids (PUFA) in wild krill that had been feeding on copepods. When Stübing *et al.* (2003) offered calanoid copepods to adult krill the typical monounsaturated fatty acids (MUFA) of the prey were not incorporated in the krill lipids, but high concentrations were found in the faecal pellets. Other studies also could not detect dietary signature fatty acids of copepods (Cripps & Hill 1998) or rotifers and *Artemia* (Alonzo *et al.* 2003, 2005a, 2005b) in krill.

Evidence that krill feed on phytoplankton during the day and switch to carnivory at night is quite strong from field observations. Hernández-León *et al.* (2001) analysed gut fluorescence and gut contents of krill during a diel cycle and reported a strong diel feeding behaviour. Both Hernández-León *et al.* (2001) and Atkinson *et al.* (1999) report that krill exert a strong top down effect on plankton community structure. Atkinson & Snýder (1997) reported that both protozoans and copepods supplied most of the required carbon in low algal biomass areas and that they selected larger copepod species, ranging in length from 1–3 mm, at faster rates than smaller copepods. When krill were given a choice between phytoplankton and copepods the copepods were cleared faster (Atkinson & Snýder 1997). Pakhomov *et al.* (1997) reported that phytoplankton intake alone could not meet the respiratory costs for krill in the South Georgia area in summer.

Understanding the dynamics of trophic interactions is fundamental to the development of ecosystem models. Present state-of-the-art modelling now helps describe the
effects on resources between and within trophic levels. Quantitative data on levels of krill herbivory versus carnivory will help to clarify community structure in terms of resources available for primary and secondary consumers. Quantitative and qualitative information also provides insights into the nutrient and energy transfer through the food web and carbon export to the deeper oceans. The degree to which krill graze on algal sea ice communities in the winter will, in turn, affect spring seeding capacity and summer algal biomass. The models being developed through the Southern Ocean GLOBEC program explicitly allow simulation of changes in biochemical composition of the organism as it develops. These developments call for a clarification of the degree of herbivory, omnivory and carnivory in krill and the effects of these feeding behaviours on condition and reproduction.

We present experimental data on krill carnivory in terms of lipid content and composition, and also outline the current state of knowledge and highlight key areas for future research on the dietary preferences of *E. superba*.

Material and methods

Live Antarctic krill were collected using a Rectangular Midwater Trawl (RMT) (Baker *et al.* 1973) in Prydz Bay (65°23’S, 80°13’E) on 5 February 2004 by RSV *Aurora Australis*. Krill were evenly distributed in seven 200–250 litre tanks in cold laboratories and seawater was continuously supplied using the ship's clean experimental seawater system whilst the vessel was south of the Polar Front. Once the seawater temperature exceeded 2.5°C the continuous water supply was closed and 50% of the water in each tank was manually replaced by chilled seawater (0.5°C) each day. Krill were kept in the dark until their return to the Australian Antarctic Division's Kingston headquarters on 13 February 2004. After their return, krill were transferred to a 2000 litre tank and acclimated to the aquarium conditions (King *et al.* 2003). These specimens were kept in the dark at 0.5°C, and served as a stock population for the experiments.

Two experimental tanks were set up to keep krill in different feeding regimes, and each tank contained 100 specimens at the start of the experiment. The experiments were carried out over nine months, with the temperatures maintained at 1°C throughout that time. Feeding regimes were: Tank A) 1–2 mg CL\(^{-1}\) of the diatom Phaeodactylum tricornutum, and B) 1–2 mg CL\(^{-1}\), which included *P. tricornutum* and pellets/minced clam. For Tank B 50 mg of pellets (Progression 2, Saltcreek Inc.) contained the following ingredients: Plankton meal, squid meal, protein powder premix, algae meal, purified fish oils, vegetable oil (source of 18:2\(\omega_6\) & 18:3\(\omega_3\)), lecithin, torula yeast, minerals, vitamin C (phosphate), cholesterol, vitamins, astaxanthin, ethoxyquin. Typical analysis of the pellets was 45% protein (min), 3% fibre (max), 7% moisture (max), 1.3% DHA, 15% lipid (min), 13% ash (max) and 1.2% EPA. Krill were fed on these pellets two or three times per week for the first three and a half months. Krill were then switched to 100 mg of minced clams five times per week for the last four and a half months of the experiment. Krill that were bred in captivity for the first time in the Port of Nagoya Aquarium had been fed clams, suggesting that this is a useful animal-based supplement (Hirano *et al.* 2003). Pellets were replaced by minced clams because a relatively high mortality was observed in tank B, when pellets were supplied.

Krill were kept in the dark for six weeks then light (1000 lux) was introduced. Krill were then maintained in 24 hour light conditions until the end of the experiment. Moults and dead animals were checked and removed daily. Fifteen animals were sampled and frozen for lipid analysis at the start of the experiment, and 3–15 animals were sampled from each tank after 1, 3, 7, and 9 month intervals. Standard length 1 (Morris *et al.* 1988) was used for length measurement.

Dry mass (DM) was determined after lyophilization for 48 h and total lipids were extracted by using a modified Bligh & Dyer (1959) procedure (Virtue *et al.* 1993a). The lipid content was determined gravimetrically and expressed as total lipid mass (mg) and in percent of dry mass (%DM). Lipid class compositions were analysed on an Iatroscan Mark V thin-layer chromatograph with flame-ionisation detector (Volkman & Nichols 1991). Fatty acid methyl esters (FAME) were prepared from total lipid extracts by transesterification in methanol containing concentrated hydrochloric acid and chloroform (10/1/1, v/v/v, 2 h at 100°C). FAME were analysed with an Agilent 6890 gas chromatograph (GC) on a 50 m x 0.32 mm i.d. fused silica capillary column (film thickness: 0.25 μm, liquid phase: HP5), with confirmation of component identification by GC-mass spectrometry as described in Phleger *et al.* (2005).

Statistics

To determine whether there was a significant difference in lipids of the three tissues after nine months on the two diets, a 2-factor nested analysis of variance (ANOVA; SPSS, version 13) was used to compare each dataset. The linear model used to examine differences in the lipids in tissues between diets was: \[ Y_{ij} = \beta_0 + \beta_1 D_i + \beta_2 T(D)_{j(i)} + e_{ij} \] where \( Y_{ij} \) is the variable under consideration (e.g. TAG, 16:1o7c), \( \beta_0 \) is the overall mean, \( \beta_1 D_i \) is the effect of the tissue nested within site and \( \beta_2 T(D)_{j(i)} \) represents individual error associated with the krill themselves. The assumptions underlying the use of Nested ANOVA are that the data are normally distributed and that there is no direct relationship between the variances and the means. For the lipid class analyses \( \log_{10}(x+1) \) transformation was required before the data met these assumptions, while no transformations were needed for the fatty acid analyses.
Results

Analysis of krill sampled over the whole experiment (day 0, month 1, 3, 7 and 9 sampling intervals) revealed no significant differences in the mean biomass of krill from time 0 (48 mg) to time nine months (49 mg). In terms of size (total length) and lipid content of krill, there were also no significant changes in mean measurements over the experimental period. There were no significant differences in the total length from time 0 (30 mm) to time nine months (31 mm) and total lipid levels from time 0 (5 mg g\(^{-1}\)) to time 9 months (8 mg g\(^{-1}\)). As a result of these data, we report only the animals analysed at the end of the experiment, and any differences between tissue and diet.

The proportions of lipid classes extracted from the tissues of krill fed on the two diets after nine months are shown in Fig. 1. Nested ANOVA revealed statistically significant differences between the two diet treatments for three of the lipid classes. The tissues of krill fed only on *Phaeodactylum tricornutum* were higher in sterol esters/wax esters (SE/WE) \((F = 7.0; P = 0.018)\), and polar lipids (PL) \((F = 7.2; P = 0.017)\), but lower in triacylglycerols (TAG) \((F = 7.9; P = 0.013)\). Amongst the tissues between the two diets, krill fed only on *P. tricornutum* had lower sterols/diacylglycerols (ST/DAG) in the hepatopancreas than in the abdomen and the other tissues, while free fatty acids (FFA) and SE/WE were low in all tissues.

Fig. 1. Lipid class composition (percentage of the total lipid) in *Euphausia superba* after nine months fed on diets of *Phaeodactylum tricornutum* and *P. tricornutum* plus pellets/clams. Body parts analysed include the abdomen, hepatopancreas and the rest (remaining body portions). SE/WE: Sterol ester/Wax ester, TAG: triacylglycerol, FFA: Free fatty acid, ST/DAG: Sterol/diacylglycerol, PL: Polar lipid, (mean + SE).

Krill fed on pellets/clams had significantly higher TAG levels (and correspondingly lower PL levels) in all body parts analysed including the abdomen, hepatopancreas and the ‘rest’ (remaining body portions). Similar TAG levels were found in the abdomen (25 ± 1.2%) and hepatopancreas (25 ± 3.1%) of clam fed krill, and up to 40 ± 1.4% in the ‘rest’. In krill fed on *P. tricornutum* TAG levels found in the abdomen and hepatopancreas were 16 ± 6.7% and 16 ± 9.5% respectively and 27 ± 7.4% in the ‘rest’. Significantly higher levels of polar lipid were found in all body parts of krill fed on phytoplankton only, ranging from 67–77% (Fig. 1).

More than 25 different fatty acids were recovered from the krill tissues in the present experiment. Only the results of the dominant six are shown in Fig. 2. Krill on the mixed diatom and pellet/clam diet had significantly higher relative levels of 16:1\(_{\omega7c}\) \((F = 7.1; P = 0.017)\) and lower relative levels of docosahexaenoic acid (DHA) \((F = 11.5; P = 0.004)\), with the hepatopancreas showing significantly lower levels of DHA than the abdomen. There were no significant effects for the other fatty acids.

Discussion

Although krill fed on pellets/clams in this study did not show any increase in size or total lipid over the experimental period, they had significantly higher TAG
levels in all body parts suggesting a greater ability to store excess energy. This storage lipid may allow the krill to sustain longer periods on a reduced dietary intake, or provide metabolic energy for maturation and reproduction. The addition of animal feed (clams) to the diet and controlled photoperiod conditions were considered important factors in the successful maturation and spawning of captive krill in Nagoya (Hirano et al. 2003). Polar lipids have been traditionally viewed as structural lipids in biomembranes, whereas neutral lipids were generally thought to have a storage or related role. Recent studies have highlighted the storage role of a specific phospholipid, phosphatidylcholine (lecithin), in some crustacean species, including krill (Hagen et al. 1996, Mayzaud 1997, Stübing 2004), although the ecophysiological advantages of phospholipid storage in euphausiids are still not understood.

Fatty acid profiles of diatom and clam fed krill had higher 16:1ω7c levels compared to diatom only fed krill despite the fact that P. tricornutum is high in this fatty acid (24%) (Virtue et al. 1993b) compared with the clam used in the present study (4%) (unpublished data). In previous studies, 16:1ω7c was found in high levels in the TAG fraction (20%) compared to the PL fraction (5%) in the lipid of krill fed on a monoculture of P. tricornutum (Virtue et al. 1993a). As clam fed krill in the present study had higher TAG levels than algal fed krill, this would conversely skew the 16:1ω7c signature.

The mixed diet - diatom and clam-fed krill - had significantly lower levels of DHA despite the clams themselves having high levels of DHA (19%) (unpublished data) compared to P. tricornutum (2.7%) (Virtue et al. 1993b). P. tricornutum, although low in DHA, has 37% EPA compared to the clam (15% EPA). When comparing fatty acid profiles of wild krill to krill that had been starved for four months, krill maintained a constant level of these long chain fatty acids (Virtue et al. 1993a). Results from the present study would imply that some degree of metabolic retroconversion of DHA is occurring in krill to sustain sufficient omega 3 levels.

A detailed set of feeding trials with a range of microalgal species and the same microalgae fed to rotifers and brine shrimp (Artemia) were conducted by Alonzo et al. (2003, 2005a, 2005b). The microalgae were selected to provide very different fatty acid (FA) profiles (e.g. diatom high in EPA, dinoflagellate and thraustochytrid high in DHA, cryptomonad high in 18:1ω9c and 18:4ω3). Krill were held individually and were fed for periods up to 20 days. Lipid content, lipid class and FA composition were analysed for hepatopancreas and whole krill. Changes detected in the whole krill were largely associated with the lipid changes observed for the hepatopancreas. In comparison to the increasing krill hepatopancreas lipid content with increasing concentration of the feed algae, the hepatopancreas of krill fed rotifers or brine shrimp showed a low lipid content. Of particular interest was that the two zooplankton diets also induced no significant differences in FA composition in relation to the species of zooplankton used (rotifer or brine shrimp) or to the species of algae consumed by these prey (Alonzo et al. 2005a, 2005b). The zooplankton diets were chosen for their ease of culture as they are commonly used by the aquaculture industry. As such they are therefore not representative of krill diet in the wild. Notwithstanding this, it is noteworthy that hepatopancreas of krill fed these two zooplankton diets could be distinguished from the phytoplankton fed krill (Alonzo et al. 2003). This key finding is suggestive that the signature lipid approach may be able to provide insight into the role of carnivory in both laboratory-reared and wild harvested krill.

Several field-based studies have pointed to the importance of copepods for krill. Perissinotto et al. (2000) showed a contribution of heterotrophic material to the diet and energy budget of Antarctic krill. This was based on measurement of total carbon content of stomachs and gut-pigment determination. The heterotrophic component was estimated to account for 17–99% of the mass of the gut contents, higher than previously believed. Granéli et al. (1993) found that krill incubated in a mix of naturally occurring phytoplankton and copepods would prey selectively on copepods until they had virtually eliminated them. Cripps et al. (1999) observed for analyses of field specimens of krill at South Georgia that one group of animals having the lowest concentrations of total fatty acids, were surviving on the lowest algal biomass in the region, and had probably resorted to carnivory on PUFA-rich copepods. A recent investigation using a range of methods on field caught krill, including stomach content, fatty acid and stable isotope analysis, showed that high growth rates of krill were attributed to a strong heterotrophic component in the diet. However, protozoans rather than copepods were the heterotrophic food source in these fast growing krill (Schmidt et al. 2006).

Against the evidence noted above, only a limited number of feeding trials have been performed for E. superba fed copepods. Stübing et al. (2003) fed a mixed copepod assemblage to krill, and compared this diet to three different diets (mixed phytoplankton, mixed ice algae, the ice diatom Fragilariopsis cylindrus). Little variation occurred in lipid content, lipid classes and fatty acid composition for juvenile and adult krill fed the different diets. However, all adults and juveniles lost biomass over the feeding period, suggesting a less than ideal feeding regime or experimental set up may have been used.

Results from this long term feeding study indicate that the addition of pellets/clams to the diet of krill improved condition (increase of storage lipids), which suggests that some degree of carnivory may aid krill growth in the field. There is evidence to suggest that a mixed diet is also advantageous to the health of krill. However, further laboratory experimentation is needed to investigate the role of carnivory in krill. Following developments over the past
decade in culturing krill, several laboratories are now capable of conducting controlled krill feeding studies on a larger scale. Results from a range of previous investigations and the present study indicate that future research on the role of carnivory in krill should consider, where possible, the use of diets representative of those available in the wild (and care taken to avoid cannibalism), and light/temperature regimes designed to reflect natural krill habitat.

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